The polymer molecules used in both the acylation and alkylation approaches may be selected from among water soluble polymers as described above. The polymer selected should be modified to have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, preferably, so that the degree of polymerization may be controlled as provided for in the present methods. An exemplary reactive PEG aldehyde is polyethylene glycol propionaldehyde, which is water stable, or mono C1-C10 alkoxy or aryloxy derivatives thereof (see, U.S. Patent Number 5,252,714). The polymer may be branched or unbranched. For the acylation reactions, the polymer(s) selected should have a single reactive ester group. For the present reductive alkylation, the polymer(s) selected should have a single reactive aldehyde group. Generally, the water soluble polymer will not be selected from naturally-occurring glycosyl residues since these are usually made more conveniently by mammalian recombinant expression systems. The polymer may be of any molecular weight, and may be branched or unbranched.

A particularly preferred water-soluble polymer for use herein is polyethylene glycol. As used herein, polyethylene glycol is meant to encompass any of the forms of PEG that have been used to derivatize other proteins, such as mono-(C1-C10) alkoxy- or aryloxy-polyethylene glycol.

In general, chemical derivatization may be performed under any suitable condition used to react a biologically active substance with an activated polymer molecule. Methods for preparing pegylated GDNF protein or variant will generally comprise the steps of (a) reacting a GDNF protein or variant with polyethylene glycol (such as a reactive ester or aldehyde derivative of PEG) under conditions whereby the protein becomes attached to one or more PEG groups, and (b) obtaining the reaction product(s). In general, the optimal reaction conditions for the acylation reactions will be determined case-by-case based on known parameters and the desired result. For example, the larger the ratio of PEG:protein, the greater the percentage of poly-pegylated product.

Reductive alkylation to produce a substantially homogeneous population of mono-polymer/GDNF protein (or variant) conjugate molecule will generally comprise the steps of: (a) reacting a GDNF protein or variant with a reactive PEG molecule under reductive alkylation conditions, at a pH suitable to permit selective modification of the a-amino group at the amino terminus of said GDNF protein or variant; and (b) obtaining the reaction product(s).

For a substantially homogeneous population of mono-polymer/GDNF protein (or variant) conjugate molecules, the reductive alkylation reaction

conditions are those which permit the selective attachment of the water soluble polymer moiety to the N-terminus of GDNF protein or variant. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the a-amino group at the N-terminus (the pKa being the pH at which 50% of the amino groups are protonated and 50% are not). The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired (i.e., the less reactive the N-terminal a-amino group, the more polymer needed to achieve optimal conditions). If the pH is higher, the polymer:protein ratio need not be as large (i.e., more reactive groups are available, so fewer polymer molecules are needed). For purposes of the present invention, the pH will generally fall within the range of 3-9, preferably 3-6.

Another important consideration is the molecular weight of the polymer. In general, the higher the molecular weight of the polymer, the fewer polymer molecules may be attached to the protein. Similarly, branching of the polymer should be taken into account when optimizing these parameters. Generally, the higher the molecular weight (or the more branches) the higher the polymer:protein ratio. In general, for the pegylation reactions contemplated herein, the preferred average molecular weight is about 2 kDa to about 100 kDa. The preferred average molecular weight is about 5 kDa to about 50 kDa, particularly preferably about 12 kDa to about 25 kDa. The ratio of water-soluble polymer to GDNF protein or variant will generally range from 1:1 to 100:1, preferably (for polypegylation) 1:1 to 20:1 and (for monopegylation) 1:1 to 5:1.

Using the conditions indicated above, reductive alkylation will provide for selective attachment of the polymer to any GDNF protein or variant having an a-amino group at the amino terminus, and provide for a substantially homogenous preparation of monopolymer/GDNF protein (or variant) conjugate. The term "monopolymer/GDNF protein (or variant) conjugate" is used here to mean a composition comprised of a single polymer molecule attached to a molecule of GDNF protein or GDNF variant protein. The monopolymer/GDNF protein (or variant) conjugate preferably will have a polymer molecule located at the N-terminus, but not on lysine amino side groups. The preparation will preferably be greater than 90% monopolymer/GDNF protein (or variant) conjugate, and more preferably greater than 95% monopolymer/GDNF protein (or variant) conjugate, with the

remainder of observable molecules being unreacted (i.e., protein lacking the polymer moiety).

For the present reductive alkylation, the reducing agent should be stable in aqueous solution and preferably be able to reduce only the Schiff base formed in the initial process of reductive alkylation. Preferred reducing agents may be selected from sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane and pyridine borane. A particularly preferred reducing agent is sodium cyanoborohydride. Other reaction parameters, such as solvent, reaction times, temperatures, etc., and means of purification of products, can be determined case-by-case based on the published information relating to derivatization of proteins with water soluble polymers (see the publications cited herein).

C. GDNF Protein Product Pharmaceutical Compositions

GDNF protein product pharmaceutical compositions typically include a therapeutically effective amount of a GDNF protein product in admixture with one or more pharmaceutically and physiologically acceptable formulation materials. Suitable formulation materials include, but are not limited to, antioxidants, preservatives, coloring, flavoring and diluting agents, emulsifying agents, suspending agents, solvents, fillers, bulking agents, buffers, delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. For example, a suitable vehicle may be water for injection, physiological saline solution, or artificial CSF, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles.

The primary solvent in a vehicle may be either aqueous or non-aqueous in nature. In addition, the vehicle may contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the vehicle may contain still other pharmaceutically-acceptable excipients for modifying or maintaining the rate of release of GDNF protein product, or for promoting the absorption or penetration of GDNF protein product across the blood-brain barrier. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dose or multi-dose form or for direct continuous or periodic infusion from an implanted pump.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or in a form, e.g., lyophilized, requiring reconstitution prior to administration.

The optimal pharmaceutical formulation will be determined by one skilled in the art depending upon the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, the disclosure of which is hereby incorporated by reference. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the present proteins and derivatives.

Other effective administration forms, such as slow-release formulations, inhalant mists, orally active formulations, or suppositories, are also envisioned. For example, the GDNF protein product pharmaceutical composition may be formulated for parenteral administration, e.g., by intracerebroventricular infusion or injection. Such parenterally administered therapeutic compositions are typically in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the GDNF protein product in a pharmaceutically acceptable vehicle. One preferred vehicle is physiological saline. The GDNF protein product pharmaceutical composition also may include particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes to provide sustained release characteristics. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation.

It is also contemplated that certain formulations containing GDNF protein product are to be administered orally. For example, GDNF protein product which is administered in this fashion may be encapsulated and may be formulated with or without those carriers customarily used in the compounding of solid dosage forms. The formulation may be designed to release the active portion of the pharmaceutical composition at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients may be included to facilitate absorption of GDNF protein product. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

D. Administration of GDNF Protein Product

The GDNF protein product may be administered topically or parenterally via a subcutaneous, intramuscular, intravenous, transpulmonary, transdermal, intrathecal, intracerebral or intraocular route. For example, one route of administration is subcutaneously or intramuscularly close to the site of injury or degeneration, at a dose of about 1 μg/kg to 1 mg/kg delivered at intervals ranging from weekly to daily. To achieve the desired dose of GDNF protein product, repeated daily or less frequent injections may be administered, or GDNF protein product may be infused continuously or periodically from a constant- or programmable-flow implanted pump. The frequency of dosing will depend on the pharmacokinetic parameters of the GDNF protein product as formulated, and the route of administration.

In cases where sensory neuropathy is caused by trauma, GDNF protein product may also be administered via a carrier means, such as a biodegradable material, containing GDNF protein product that may be surgically implanted at or near the cite of injury. It is also contemplated that such a carrier composition may be packed within a wound.

Regardless of the manner of administration, the specific dose is typically calculated according to body weight or body surface area. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them, especially in light of the dosage information and assays disclosed herein. Appropriate dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data.

The final dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g., the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels for the treatment of various diseases and conditions.

It is envisioned that the continuous administration or sustained delivery of GDNF may be advantageous for a given treatment. While continuous administration may be accomplished via a mechanical means, such as with an infusion pump, it is contemplated that other modes of continuous or near

continuous administration may be practiced. For example, chemical derivatization may result in sustained circulation or sustained release forms of the GDNF protein which have the effect of continuous presence in the blood stream, in predictable amounts, based on a determined dosage regimen. Thus, GDNF protein products include proteins derivatized to effectuate such continuous administration.

GDNF protein product cell therapy, e.g., implantation of cells producing GDNF protein product, is also contemplated. This embodiment would involve implanting into patients cells capable of synthesizing and secreting a biologically active form of GDNF protein product. Such GDNF protein product-producing cells may be cells that are natural producers of GDNF protein product (analogous to B49 glioblastoma cells) or may be recombinant cells whose ability to produce GDNF protein product has been augmented by transformation with a gene encoding the desired GDNF protein product. Such transformation may be accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered GDNF protein product of a foreign species, it is preferred that the natural cells producing GDNF protein product be of human origin and produce human GDNF protein product. Likewise, it is preferred that the recombinant cells producing GDNF protein product be transformed with an expression vector containing a gene encoding a human GDNF protein product. Implanted cells may be encapsulated to avoid infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in biocompatible, semipermeable polymeric enclosures or membranes that allow release of GDNF protein product, but that prevent destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce GDNF protein product *ex vivo*, could be implanted directly into the patient without such encapsulation.

GDNF protein product gene therapy *in vivo* is also envisioned, by introducing the gene coding for GDNF protein product into targeted cells via local injection of a nucleic acid construct or other appropriate delivery vectors. (Hefti, *J. Neurobiol.* 25:1418-1435, 1994). For example, a nucleic acid sequence encoding a GDNF protein product may be contained in an adeno-associated virus vector for delivery into the targeted cells. Alternative viral vectors include, but are not limited to, retrovirus, adenovirus, herpes

simplex virus and papilloma virus vectors. Physical transfer may also be achieved by liposome-mediated transfer, direct injection (naked DNA), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation or microparticle bombardment (gene gun).

The methodology for the membrane encapsulation of living cells is familiar to those of ordinary skill in the art, and the preparation of the encapsulated cells and their implantation in patients may be accomplished without undue experimentation. See, e.g., U.S. Patent Numbers 4,892,538, 5,011,472, and 5,106,627, each of which is specifically incorporated herein by reference. A system for encapsulating living cells is described in PCT Application WO 91/10425 of Aebischer et al., specifically incorporated herein by reference. See also, PCT Application WO 91/10470 of Aebischer et al., Winn et al., *Exper. Neurol.*, 113:322-329, 1991, Aebischer et al., *Exper. Neurol.*., 111:269-275, 1991; Tresco et al., *ASAIO*, 38:17-23, 1992, each of which is specifically incorporated herein by reference.

It should be noted that the GDNF protein product formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples. Example 1 addresses the use of radiolabelled GDNF protein product to show that neonatal and adult sensory neurons bind, internalize and retrogradely transport GDNF in a receptor-mediated fashion. Example 2 addresses the effect of GDNF protein product administration in a rat sensory neuron injury model.

EXAMPLES

Example 1
Retrograde Transport of GDNF Protein Product in Neonatal and Adult Sensory Neurons

In this experiment, ^{125}I-radiolabelled GDNF protein product was used to show that neonatal and adult sensory neurons bind, internalize and retrogradely transport GDNF protein product in a receptor-mediated fashion.

The GDNF protein product used in Examples 1 and 2 was recombinant human [Met^{-1}] GDNF and was produced by expression in *E. coli* as generally described in Examples 6B and 6C of WO93/06116. The purified [Met^{-1}]GDNF was iodinated using the lactoperoxidase technique and separated from free ^{125}I using G-25 Sephadex quick spin columns as described in Yan et al., *J. Neurobiol.*, *24*:1555-77, 1993. Labeled ^{125}I-[Met^{-1}]GDNF in 2µl of PBS (19.87 ng/µl; 2.1x10^{6} cpm/µl), with or without a 111-fold excess of unlabeled [Met^{-1}]GDNF, was injected into the right footpad of the hindlimb of neonatal rats (n=12) (P1) or into the sciatic nerve of adult rats (n=12). The animals were allowed to recover and survive for 16 hours. L4 and L5 dorsal root ganglia were dissected free from surrounding tissues and counted in a scintillation counter. These dorsal root ganglia were further processed for emulsion autoradiographs.

After injection into the hindlimb of neonatal rats, the ^{125}I-[Met^{-1}]GDNF was retrogradely transported by L4 and L5 dorsal root ganglia ipsilateral to the injection side, as determined by direct measurement of transported radioactivity in the dissected L4 and L5 dorsal root ganglia. The specificity of this transport was demonstrated by the much higher accumulation of radioactivity in ipsilateral spinal cords. Cell sizing histograms of autoradiograms indicated that both large and small neurons take up GDNF protein product in neonatal rats. No silver grains above background were observed in the contralateral dorsal root ganglia. Co-injection of an 111-fold excess of unlabeled [Met^{-1}]GDNF completely blocked transport of ^{125}I-[Met^{-1}]GDNF in the ipsilateral side, indicating that the retrograde transport of [Met^{-1}]GDNF was saturable and receptor-mediated. These results show that dorsal root ganglia neurons can bind, internalize, and retrogradely transport GDNF protein product in a specific, receptor-mediated fashion.

In adult rats, injection of ^{125}I-[Met^{-1}]GDNF into the sciatic nerve also resulted in a receptor-mediated retrograde transport to lumbar dorsal root ganglia neurons, as determined by direct measurement of transported radioactivity in the dissected dorsal root ganglia. Cell sizing histogram examination of autoradiograms indicated that the large sensory neurons preferentially take up GDNF protein product in adult rats.

Retrograde transport of a neurotrophic factors is significant because these factors initiate their effects by binding to cell surface receptor followed by uptake and retrograde transport to the cell body. The specific retrograde transport of GDNF protein product by dorsal root ganglia neurons indicates that

these neurons express a GDNF receptor and that GDNF may play a physiological role as a target-derived neurotrophic factor for these neurons.

Example 2

Promotion of Sensory Neuron Survival via Administration of GDNF Protein Product

In this experiment, the activity of GDNF protein product was tested in a rat sciatic nerve axotomy model. A reproducible cell death occurs after axotomy in the neonatal period (Snider et al., *J. Neurobiol.*, *23*:1231-46, 1992), making such models ideal systems for assessing the survival-promoting activity of factors for given neuronal populations. Axotomy of the sciatic nerve in neonatal rats results in the death of approximately 40-50% of the sensory neurons in L4 and L5 dorsal root ganglia after 7 days (Himes and Tessler, *J. Comp. Neurol.*, *284*:215-30, 1989; Yip et al., *J. Neurosci.*, *4*:2986-92, 1984).

Briefly, newborn (P0) rats were pretreated with 25 µg of [Met^{-1}]GDNF (1 mg/ml) or cytochrome c as a negative control via subcutaneous injection into the right thigh. On P1, the animals were anesthetized by hypothermia. The right sciatic nerve was cut near the obturator tendon and a 3x3x3 mm^3 piece of Gelfoam soaked in 1 mg/ml [Met^{-1}]GDNF was implanted at the cut nerve stump. On P4, P5, and P6, an additional 5 µl of [Met^{-1}]GDNF (1 mg/ml) was injected into the Gelfoam-implanted site. The animals were sacrificed on P7 and their L5 dorsal root ganglia were processed for paraffin section histology. Sections were coded to prevent bias and the neurons with clear nuclei and distinct nucleoli were counted.

In the animals receiving cytochrome c treatment as a negative control, only 58% of the neurons survived in the L5 dorsal root ganglia at 7 days after axotomy. In animals treated with GDNF protein product, 98% of L5 dorsal root ganglia neurons survived at 7 days after axotomy. These results show that GDNF protein product administration protects almost 100% of sensory neurons from axotomy-induced cell death in neonatal rats.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the present invention are those which appear in the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Yan, Qiao
Matheson, Christine R.

(ii) TITLE OF INVENTION: Method for Treating Sensory Neuropathy Using Glial Cell Line-Derived Neurotrophic Factor (GDNF) Protein Product

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: United States of America
(F) ZIP: 91320

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Curry, Daniel R.
(B) REGISTRATION NUMBER: 32,727
(C) REFERENCE/DOCKET NUMBER: A-361

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 805-499-8011
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: inferred amino acid sequence for mature human GDNF

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ser Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu Arg Asn Arg
1 5 10 15

Gln Ala Ala Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg
20 25 30

Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu
35 40 45

Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile
50 55 60

Phe Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Thr Tyr Asp
65 70 75 80

Lys Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val Ser Asp Lys
85 90 95

Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp Asp Leu Ser
100 105 110

Phe Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys His Ser Ala
115 120 125

Lys Arg Cys Gly Cys Ile
130

CLAIMS

What is claimed is:

1. The use of a glial cell line-derived neurotrophic factor (GDNF) protein product for the manufacture of a pharmaceutical composition for the treatment of sensory neuropathy.

2. The use according to claim 1 wherein the sensory neuropathy is a secondary complication of a non-neurological condition.

3. The use according to claim 2 wherein the sensory neuropathy is a secondary complication of trauma.

4. The use according to any of claims 1 to 3 wherein the pharmaceutical composition comprises a GDNF amino acid sequence set forth in SEQ ID NO:1 or a variant or a derivative thereof.

5. The use according to claim 4 wherein the pharmaceutical composition is [Met^{-1}]GDNF.

6. The use according to claim 4 wherein the derivative comprises a water soluble polymer.

7. The use according to any of claims 1 to 3 wherein the pharmaceutical composition is a sustained-release pharmaceutical composition.

8. The use according to any of claims 1 to 3 wherein the pharmaceutical composition comprises cells have been modified to produce and secrete the GDNF protein product.

9. The use according to any of claims 1 to 3 wherein the pharmaceutical composition further comprises an effective amount of a second therapeutic agent for treating sensory neuropathy.

10. The use according to claim 9 wherein the second therapeutic agent is selected from the group consisting of nerve growth factor (NGF), neurotrophin-3 (NT-3), and insulin growth factor-I (IGF-I).

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INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 96/18729

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 06116 A (SYNTEX SYNERGEN NEUROSCIENCE J) 1 April 1993 cited in the application see page 37, line 26 - page 41, line 22 see page 43, line 5 - line 17	1-10
X	WO 95 17203 A (UNIV MEDICINE AND DENTISTRY OF) 29 June 1995 see page 18, line 8 - page 20, line 23	1,9,10
X	WO 93 15722 A (SYNTEX INC) 19 August 1993 see page 4, line 24 - page 6, line 37	1,7
X,P	WO 96 14861 A (AMGEN BOULDER INC ;MARTIN DAVID (US)) 23 May 1996 see page 4, line 15 - page 8, line 27	1,7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 April 1997	10.04.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Rempp, G

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/18729

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 401 384 A (KIRIN AMGEN INC) 12 December 1990 cited in the application ---	7
A	PATENT ABSTRACTS OF JAPAN vol. 095, no. 007, 31 August 1995 & JP 07 101990 A (SUMITOMO METAL IND LTD), 18 April 1995, see abstract -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 96/18729

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9306116 A	01-04-93	AU 2681192 A	27-04-93
		CA 2119463 A	01-04-93
		EP 0610254 A	17-08-94
		FI 941285 A	18-03-94
		HU 66137 A	28-09-94
		HU 9500478 A	30-10-95
		JP 7502980 T	30-03-95
		NO 940922 A	19-05-94
		NZ 244392 A	28-03-95
		PT 100879 A	28-02-94
		ZA 9207159 A	02-08-93
WO 9517203 A	29-06-95	AU 1443095 A	10-07-95
WO 9315722 A	19-08-93	US 5470582 A	28-11-95
WO 9614861 A	23-05-96	AU 3752895 A	06-06-96
EP 0401384 A	12-12-90	AT 135370 T	15-03-96
		CA 2006596 A	22-06-90
		DE 68925966 D	18-04-96
		DE 68925966 T	29-08-96
		WO 9006952 A	28-06-90